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THE CULTIVATION OF *HERPETOMONAS MUSCARUM* (LEIDY 1856) KENT 1881 FROM *LUCILIA SERICATA* *

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The first attempts to cultivate herpetomonad flagellates from the house fly were made by Franchini and Mantovani (1915). They reported anaplasma-like bodies in the water of condensation of NNN medium, containing 3 per cent glucose, which they had inoculated with blood from a rat injected with the intestine of an infected fly. Two rats injected with these "cultural" forms showed Leishmania-like forms in the peritoneal fluid, but NNN medium inoculated with the peritoneal fluid of these rats, remained sterile. Glaser (1922) elaborated a complicated method for separating the flagellates from the associated bacteria, and also prepared a special medium, on which he was able to keep the flagellate alive in culture for 14 days. He described only flagellated forms in his culture. Patton, La Frenais and Sundara Rao (1921) in a brief report, stated that they used cultures of different insect flagellates in their feeding experiments with bed bugs. They mentioned *Herpetomonas mirabilis* Roubaud, from *Lucilia argyricephala; H. muscae domesticae* Burnett, from *Lucilia argyricephala;* and *Crithidia tabani* Patton, from *Haematopota* sp. No description of their method of cultivation or of the cultural forms obtained was given.

In the summer of 1923, while searching for different herpetomonad flagellates of insects, I found a large percentage of the green bottle fly, *Lucilia sericata,* infected with *Herpetomonas muscarum* (Leidy 1856) Kent 1881, the species commonly referred to as *H. muscae-domesticae.* I repeated the work of Franchini and Mantovani and of Laveran and Franchini (1920) in the inoculation of animals with this flagellate. The results were given in previously published papers (1925). At the same time I succeeded in cultivating the herpetomonad of this fly.

MATERIAL AND METHODS

Flies were caught either outside the laboratory or inside by baiting them with the dead body of a mouse or other small laboratory animal.

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They were killed by either chloroform or ether narcosis and placed in 2 per cent lysol for two to four minutes. After washing them in five or six changes of sterile saline, they were placed on a flamed cork or a glass plate. The abdomen was then opened with flamed needles, the intestine separated from the other organs and stretched out in a drop of sterile saline solution. The intestine was divided by means of needles into anterior, middle and posterior portions which were transferred into separate drops of sterile salt solution. Each portion was examined under the microscope and those found infected with flagellates were used for cultivation.

Since the fly's intestine is usually infected with bacteria, the isolation of the flagellate by means of the plating method used by Nöller (1917) was employed. Pure cultures were also obtained by the Barber technic of picking out a single organism, Chamber's micromanipulator being employed for this purpose.

It was found that the flagellate would grow on blood agar made up with a glycerin agar base, which was adjusted to p_H 6.2 The medium was prepared by mixing either rabbit or horse blood diluted with an equal amount of sterile Locke's solution, with the liquefied glycerin agar base. Either defibrinated or citrated blood may be employed in this medium. For trial of media of different hydrogen ion concentrations, the buffered saline solution was employed and adjusted to a series of p_H varying from 5.2 to 8.2. Red cells adjusted to the same concentration of p_H were added to it in the same way as described in Part III of my study on the relation of insect flagellates to leishmaniasis. When experimenting with solid media of different hydrogen ion concentration, the buffered NNN agar base was adjusted and the red cells were added to it in the same way as described also in the above mentioned paper.

During the months of July and August, 1923, I examined 300 specimens of *Lucilia sericata* and 250 of them, or 83.4 per cent were found infected with Herpetomonas. The number of flagellates in a single fly varied greatly in different individuals. In some only a few were present, whereas in others the middle portion of the intestine was swarming with them. In order to determine whether there is any evidence in support of Prowazek's view (1904) that the organisms are transmitted through the ovum, the distribution of the flagellates in the alimentary tract was studied in serial stained sections of the infected intestines (Figs. 18, 19, 20). The parasites were found to be limited to the lumen of the alimentary tract. The first portion of the intestine was the part most heavily infected and here the flagellated forms predominated. In the posterior part of the intestine and the rectum the organisms were usually less abundant and rounded forms predominated. No organisms were found in any of the cells of the intestine or in any

other organs. Particular attention was paid to the ovary. Different organs including ovary and eggs were taken from infected flies and planted on culture media, but all attempts to obtain cultures from the tissues proved negative. On one occasion a fly of the above species was found very heavily infected with Herpetomonas, and containing comparatively few bacteria. This material, appearing unusually favorable, was taken and portions of the dissected intestine were distributed on plates and Kolle flasks containing glycerin blood agar. Single flagellates were also isolated by means of the Chamber's micromanipulator, and 12 tubes were inoculated each with one organism. In order to plant these, the fine end of the pipette was broken off in the water of condensation of the culture medium. After four days small colonies of flagellates were observed on the plates between large colonies of bacteria. These colonies were then transplanted into tubes of media and in seven days the water of condensation was milky in appearance and showed a pure culture of motile flagellates.

Four of the 12 tubes, each inoculated with a single organism, furnished pure cultures at the end of the sixth day and the flagellates subsequently increased in number. These cultures were transferred on different media and have since been propagated continuously. At the time of preparing this paper the strain has reached its 45th generation and is 12 months old. The plating technic was used subsequently on several occasions so that 4 other strains from the same species of fly have been isolated.

The glycerin agar was taken as the base for preparing the blood agar, only by chance, because on the day that an unusually rich infection was obtained, the supply of NNN agar base happened to be exhausted, and glycerin agar being available was therefore employed. However, when transplants on the standard NNN medium were made, practically no growth was obtained, but when blood agar prepared with glycerin agar was again employed, a very good growth resulted. It thus appeared important to investigate the growth requirements of this flagellate. First the p_H of the water of condensation of flourishing cultures was estimated and was found to range between p_H 5.4 to 6.4, which was very low as compared with that of Leishmania cultures which grow best at the p_H of the blood, that is about p_H 7.0. Therefore a series of buffered saline solutions containing blood and adjusted to different p_H concentrations varying from 5.2 to 8.6 were inoculated with the flagellate. Later on, solid media were prepared by adding adjusted blood saline solution to the buffered NNN agar base, so that the above range of p_H concentrations resulted. Very good growth of the flagellates was obtained between 5.4 and 6.4, whereas only slight growth occurred on tubes between 6.8 and 7.2 and practically no growth

from p_H 7.6 to 8.6. This fact probably explains to a large extent the failure of other authors to cultivate this parasite. It also shows that it was the reaction of the glycerin agar employed which favored the growth of the flagellate, rather than the glycerin content.

The microscopic appearance of the cultures as well as the number and morphology of the flagellate varied characteristically in tubes of different p_H concentrations. The water of condensation of tubes of p_H 5.6 to 5.8 was milky in appearance and swarming with long attenuated forms of the flagellates, and also many "giant" forms to be described in detail further on, were present. In tubes of higher concentration the water of condensation remained clear, and in microscopical preparations from such cultures, the flagellates were usually markedly shorter. In tubes of p_H 7.8 only rare forms were present having almost always a rounded form. After determining the proper p_H for the media, the ingredients used in its preparation were taken into consideration. Planted in tubes of blood agar, the blood content of which varied from 15 per cent to 50 per cent, it was found that a concentration of 20 per cent to 25 per cent of blood was most favorable to the growth of the flagellate. Either defibrinated rabbit or citrated horse blood were used, without notable difference in the growth. Dilution of the blood was made with physiological salt solution or Locke's solution. Growth was also obtained on media without red blood corpuscles, such as coagulated serum, serum agar, or soft glycerin agar, providing the p_H concentration was between 5.4 and 6.4. The growth under such circumstances was not so abundant as on media containing whole or defibrinated blood and the parasites degenerated and disappeared much sooner.

Regarding the effect of temperature on growth the cultures were usually kept at room temperature and good growth was obtained in four to five days. In the incubator at 30 C. to 37 C., growth was more rapid, so that in 24 hours a rich culture was obtained, but degeneration under such conditions takes place sooner, so that for the stock cultures high temperatures are not practical. For keeping the cultures without the need of frequent subculturing, the best temperature is a cool room temperature of about 50 to 60 F. In general this Herpetomonas of the fly in culture tolerates higher temperatures than *H. ctenocephali* or Leishmania. The longest time that the flagellates were kept on medium without transfer was 5 months. The tubes were inoculated on November 12, 1923, and then sealed with paraffin. On April 25, 1924, motile forms were still present and successful subcultures were made.

MORPHOLOGY AND DIVISION

Kent (1881) in describing the flagellate of the house-fly mentioned one flagellum as characteristic. Subsequently many authors have

reported flagellates from the house-fly and muscoid flies, but they disagree with regard to the number of flagella present. Thus one school follows Prowazek (1904) in considering the organism as biflagellate; the French authors, especially Chatton, Alilaire and Roubaud being of this opinion, while the other school follows the views of Patton (1909) who observed that the two flagella seen so often are due to the fact that the parasite divides very rapidly and that the growth of the second flagellum precedes the division of the parabasal body and the nucleus. Most of the forms seen in culture were identical with those previously described by Patton (1909), MacKinnon (1910), Wenyon (1913), Brug (1914), and Becker (1923). In fresh cultures only flagellated forms were present, of which about 40 per cent had two flagella, which were in some instances of the same, in others of unequal length. In rather old cultures the flagellated forms became rounded up, but the flagellum does not disappear as in some herpetomonads, but is taken into the body of the rounded parasite as will be described later on. Large "giant" forms with many nuclei and parabasal bodies and possessing sometimes from three to five flagella were often seen in cultures showing an exceptionally heavy growth. Such forms were not as motile as the typical small forms.

The parasite may be considered as typically uniflagellate, since only uniflagellate forms are produced as the result of division. The first stage of division is the appearance of the second flagellum, growing out from the region of the parabasal body, along side of the original flagellum. The parabasal body and nucleus at this time are still undivided. When the second flagellum reaches a given length, the parabasal body divides. The nucleus divides after the division of the parabasal body, but this is however not a constant feature, as I have often observed individuals possessing two nuclei and two flagella arising from an undivided parabasal (Fig. 4). After the division of the nucleus the body divides and the two newly formed parasites separate. These young forms are thus invariably uniflagellate. In older cultures the parasite assumes a rounded form and at the same time the parabasal body which is usually anterior to the nucleus, travels toward the nucleus or it may even come to lie posterior to it (Figs. 8, 10, 11). During this change of position the parabasal body draws the flagellum with it. In this way crithidia and trypanosoma-like forms appear which are however devoid of an undulating membrane. These forms usually are not present in young cultures, in which typical flagellates with anteriorly situated parabasal bodies predominate.

On one or two occasions a line passing to the posterior end of the organism was seen in stained films (Fig. 14) which would correspond to Prowazek's "Doppelfaden" or Leger's "intestinal canal." Parasites

were seen without nuclei and with only parabasal bodies (Figs. 12, 13). These were described by Prowazek as male forms. However upon the examination of the stained films, individuals were seen which had just completed division but in which the nucleus did not divide, so that one of the daughter organisms, although possessing a parabasal body was without a nucleus. This is probably the origin of the anucleate forms depicted in our microphotographs (Figs. 12, 13). These forms most likely degenerate and are probably unable to multiply.

The large forms, mentioned above, are at least ten times as large as the normal cultural forms. They are rounded, possess from 2 to 5 nuclei, and usually the same number of parabasal bodies. Flagella when present are variable in number and size (Figs. 15 to 17). These forms are probably produced by rapid nuclear division while the protoplasm fails to keep up with the process and remains undivided. This view is supported by the fact that they were chiefly present in cultures showing rapid growth. The large multinucleated forms were never observed in smears made from the contents of the fly's intestine.

Regarding the measurements of the cultural forms, considerable variation was found, as has been reported by different authors for forms in the fly's intestine. The length of the flagellum appears to be inversely proportional to the width of the individual flagellate. Thus the long flagellated forms in some cultures average about 2.5μ in width and 22.5μ in length and the flagellum itself measure about 30μ. When the flagellate becomes shorter before rounding up, the dimensions are about 5 by 15μ, later changing to 6 by 12μ and 7.5 by 9μ. The flagellum becomes shorter and is also to some extent taken into the body, so that the free portion is shortened to only 4μ. The measurements of the giant forms shown in the microphotographs are 8.5 by 13μ, 12 by 15μ, 18 by 21μ and 20 by 19.5μ when only the body without flagella was included.

NOMENCLATURE

Regarding the name of the species of herpetomonas which has been cultivated from *Lucilia sericata,* it might be considered to be either *Herpetomonas mesnili,* first described by Roubaud (1908) from *Lucilia* sp, or *Herpetomonas luciliae,* named and described by Strickland (1911) from *Lucilia* sp.

Becker (1923) has reported that *Herpetomonas muscae-domesticae* (=H. muscarum) is entozoic in various species of Lucilia and other muscoid flies. His work on the group specificity of herpetomonas of muscoid flies and similar experiments of our own, done with pure line cultures, show that the herpetomonad commonly found in *Lucilia sericata* occurs also in other flies. The fact that experimental infection was produced in three other species of muscoid flies by feeding culture

of this flagellate indicates that some of the species of Herpetomonas described in various flies should be reconsidered with a view to their possible suppression. As pointed out by Hoare (1923-1924) the species under consideration should be known as *Herpetomonas muscarum* rather than *Herpetomonas muscae-domesticae.*

SUMMARY

An herpetomonas requiring a medium of low hydrogen ion concentration, i. e., p_H 5.6 to 6.4, was isolated in pure line culture from the green bottle fly, *Lucilia sericata.* It was found in 83.4 per cent of specimens examined.

On account of the group specificity of herpetomonads obtained from muscoid flies, and from the results of Becker's and our own experiments, as well as from their morphological identity, they are considered to be of the species *Herpetomonas muscarum* (Leidy 1856) Kent 1881.

This species should be considered as uniflagellate. In division a precocious growth of the second flagellum gives the impression that the parasite is biflagellate.

Forms without nucleus found also in pure line culture may be explained on the basis of atypical division.

Large multinuclear forms, with multiple nuclei and parabasal bodies developed in cultures, being sometimes ten times as large as usual. These probably result from a failure of the cleavage of the cytoplasm to keep pace with nuclear division.

No intracellular forms of *H. muscarum* were found. The failure to find this species on microscopial examination of the ovary or in cultures inoculated with ovarian tissue, indicates that it is not commonly transmitted through the egg of the fly.

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Explanation of Plate XIX

Fig. 1.—Uniflagellate elongated form showing single flagellum.

Fig. 2.—An organism showing the early development of the second flagellum, which protrudes from the surface at the origin of the first.

Fig. 3.—A form showing second flagellum extending for about one-fifth the length of the first flagellum and united with it.

Fig. 4.—An organism showing development of the second flagellum, and beginning division of the nucleus.

Fig. 5.—The second flagellum extends for more than two-thirds the length of the original flagellum, to which it adheres.

Figs. 6 and 7.—Biflagellate forms with nucleus elongating transversely preparatory to division.

Figs. 8, 10 and 11.—Migration of the parabasal body toward the posterior extremity with inclusion of a considerable portion of the flagellum.

Fig. 9.—Biflagellate form showing division of the parabasal body and beginning division of the nucleus.

Fig. 12.—Rounded anucleate form, evidently degenerating.

Fig. 13.—Elongated and rounded forms, the larger one of the latter in process of division; anucleate form indicated by "X."

Fig. 14.—Various types of the flagellate, the one marked "X" showing an unstained fiber extending to the posterior extremity.

Fig. 15.—Two large multinucleate forms adherent to elongated organisms.

Fig. 16.—Rounded herpetomonad, showing three flagella two of which extend free from surface.

Fig. 17.—Rounded organism showing numerous nuclei and flagella.

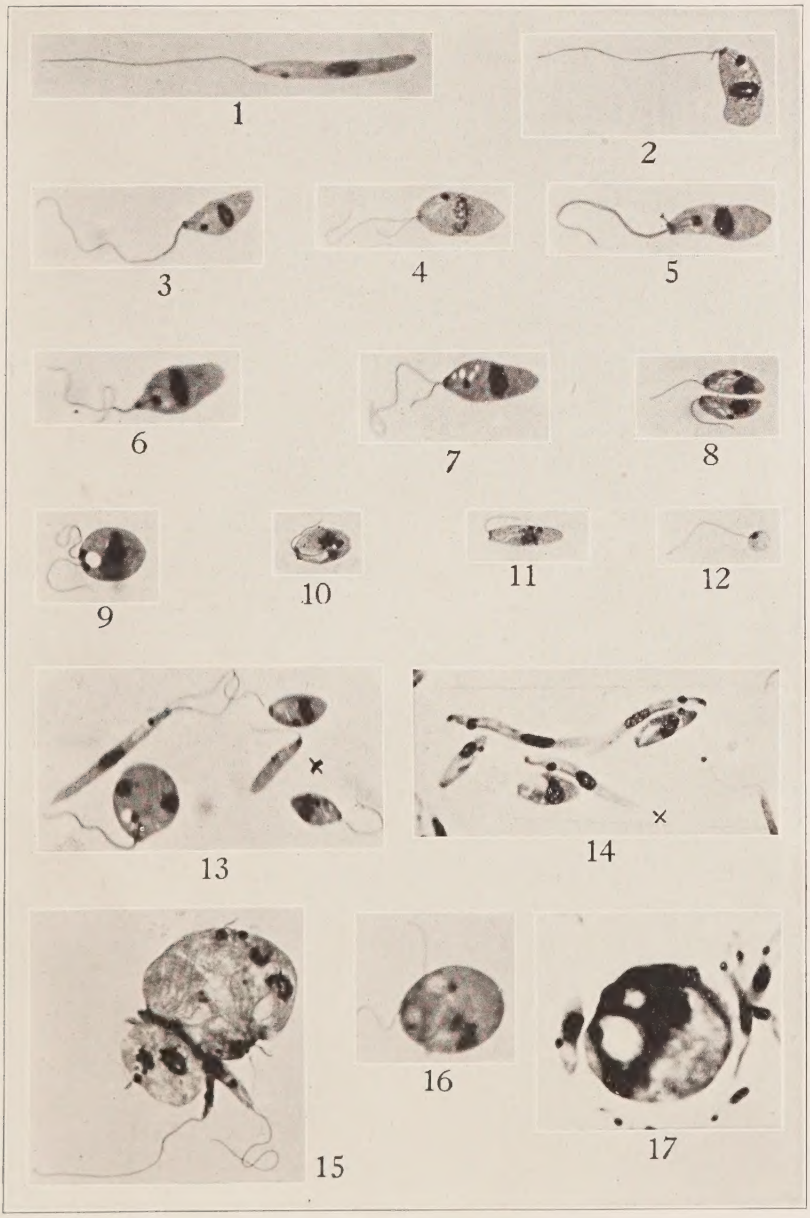


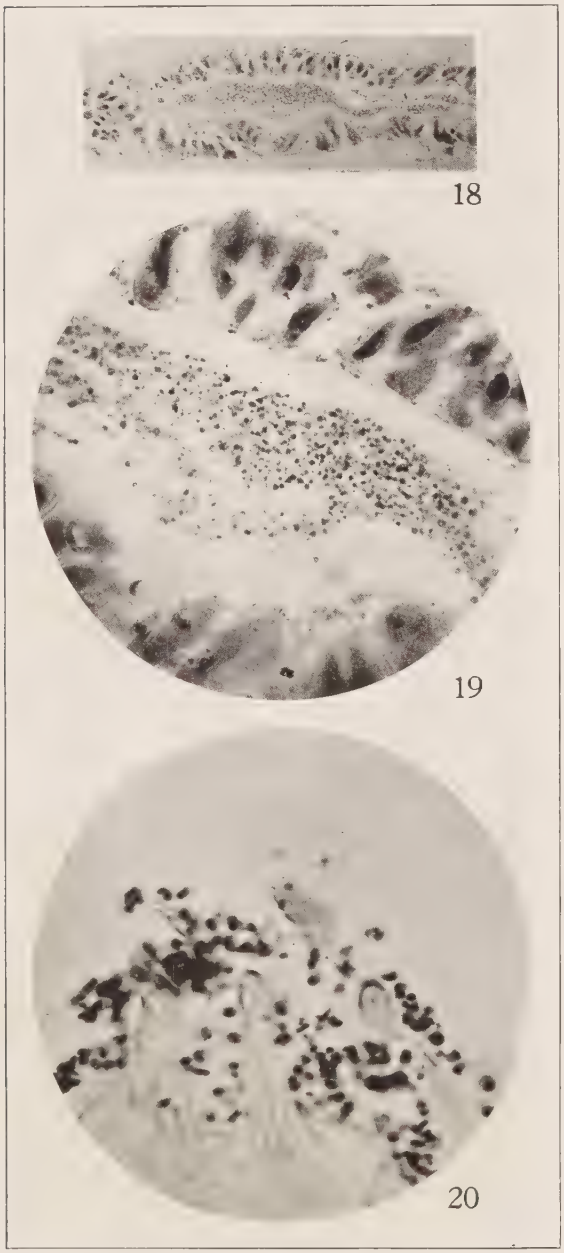
PLATE XIX

Expanation of Plate XX

Fig. 18.—Cross section of the anterior portion of the intestine of the fly, *Lucilia sericata*. Large numbers of flagellates in the lumen.

Fig. 19.—Higher magnification of same portion of the intestine shown in figure 18, the deeply staining bodies representing nuclei of the flagellates.

Fig. 20.—Stained section from the region of the rectum, showing rounded forms of the flagellate.



18

19

20

PLATE XX

ENDOLIMAX REYNOLDSI NOV. SP. FROM THE INTESTINE OF THE COMMON SWIFT, *SCELOPORUS UNDULATUS* *

CLAUDE MATTHEWS MCFALL

During the month of May, 1925, a number of southern lizards were collected in South Carolina and Georgia and their blood and intestinal contents were examined for parasites. This collection included the common swift, sand streaker, glass lizard and chameleon. Parasites were not found in the blood of any specimen examined. On the other hand the intestines of these species were heavily parasitized by various types, such as amoebae, flagellates, coccidia and worms, that afford excellent and abundant material for the student of endoparasitology.

For my own investigations *Sceloporus undulatus* was chosen for two reasons, viz., it is the most numerous of the above named species and it will survive longest in captivity without food. The intestinal contents of more than 80 of the common swifts have been examined. These came from Virginia, the Carolinas and Georgia, but the majority were obtained from South Carolina. All except five very young ones were infected with some of the four forms of parasites previously mentioned. The males have a higher infection than the females, especially during the laying season. Just why this is true, I have not been able to determine. But the very young lizards are not infected.

So far as I have been able to learn no extensive study of the intestinal fauna of the American lizard has ever been made. In Europe, Grassi, Dobell, Hartmann, Prowazek and others have furnished interesting reports on the protozoa found in some lizards in Italy and Germany. *Amoeba lacertae* was discovered by Hartmann and Prowazek in 1907 and later studied by Nägler in 1909. On account of the latter's extreme brevity and confusion in his drawings, Nägler's report leaves much to be desired.

Dobell, in 1914, near Naples, studied an amoeba from the gut of *Lacerta muralis*. His report, while differing materially from Nägler's, leaves one persuaded that they were dealing with the same species. In addition to *Amoeba lacertae*, Dobell says there is often present another form that appears to be a true parasite, an Entamoeba; but as he could not, at that time, find a single dividing form of this species, he decided not to give it a specific name. In describing the motile form he says: "Large specimens possess, when rounded, a diameter of 30μ or slightly more; very small individuals do not exceed 10μ. In the largest forms

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the diameter of the nucleus is about 6.5μ, in the smallest about 3.5μ. The nucleus contains a large karyosome, surrounded by numerous granules or peripheral chromatin supported on a linin network (Fig. 15). There is a very thin nuclear membrane present. The cytoplasm has a delicate alveolate structure in fixed specimens, and is always packed with food bodies. No contractile vacuole is present. When alive, this organism has an appearance (the nucleus excepted) very much like that of *Entamoeba ranarum,* and moves in a similar manner." Nägler also mentions another amoeba which he calls *Basidiobolus lacertae,* and which he says always accompanies *Amoeba lacertae.* I find something that has an appearance like the amoeboid form to which Nägler gave the generic name of Basidiobolus, but as I have not been able to see the motile form nor its complete stages of division, I should hesitate to call it an amoeba.

Examination for the living forms was made first by taking fecal matter from the cecum. In order to save time it is best to snip off the lizard's head with a pair of sharp curved scissors. The highest infection in starved individuals is always in the region of the cecum. The fecal matter was placed on a slide in a 0.7 per cent normal salt solution and if the organisms were present, they could be seen readily with the 16 mm. objective.

Smears were made from all parts of the large and small intestine; no egg albumin was used unless the fecal matter was insufficient to hold the organisms on to the slide. Various fixatives were used in studying this material, viz., Schaudinn's aceto-sublimate and Bouin's solution. The slides were mordanted for at least two hours, sometimes overnight. They were always left in the stain overnight and sometimes longer. Naturally it takes longer for the destaining process but one gets a much finer definition in the organisms so treated. All smears were made directly from the intestine; although one culture lived for six days in beef extract and a 0.7 per cent normal salt solution, there were never enough of the organisms in this culture to make a successful smear.

The smear method is satisfactory for the study of the flagellates, but for the amoebae fixing the entire intestine in Bouin's solution for several (16 to 18) hours proved much more desirable. This fixation was followed by Heidenhain's iron hematoxylin method of staining, after the tissue is sectioned in paraffin. The amoebae in these preparations are much more numerous because they are less likely to be washed off the slide as they so often are in the smear method. They also stain and destain to a much more desirable degree. More than a hundred slides were prepared by the smear method and the amoebae were found on 90 per cent of them, but always in very small numbers, never more than one or two in each field whereas in the sections, as high as 21 have been counted in one field under the 16 mm. objective.

THE MOTILE AMOEBA

The motile amoeba exhibits two widely different forms; the first (Fig. 1) is characterized by having an extraordinarily long, narrow pseudopodium, a diamond-shaped nuclear membrane, enclosing a thin layer of "peripheral chromatin." It is highly vacuolated but careful observation shows no food vacuoles with food inclusions. The second form is characterized by having broad, blunt pseudopodia (Fig. 11) and cytoplasm highly speckled with faintly staining bodies that cannot be regarded as food, for there are no food vacuoles present. These bodies may possibly be parasitic in nature for the nucleus in figure 11 appears to be undergoing reduction and in figure 12 it has disappeared entirely.

The average diameter, when rounded is 14.5μ. Others measured 13.2 by 20.8μ and one 15 by 45μ (Fig. 1). The smallest specimens averaged about 7.5μ. The diameter of the entire nucleus averages 4.5μ, varying from 3 to 6μ in diameter. Its structure may be considered under four heads, viz. (1) the nuclear membrane; (2) the karyosome; (3) the peripheral layer of granules, and (4) the linin threads.

The nuclear membrane is clearly visible unless the organism is filled with the problematic bodies seen in Figure 12. Where there are none of these bodies (Fig. 1) the nucleus is plainly visible. In most cases, with iron hematoxylin, a border of very fine granules can be seen just inside the nuclear membrane (Fig. 3). These may be chromatin granules but they are not as large as those characteristic of Endamoeba. The membrane is not always spherical, it is very often polyhedral in shape (Fig. 2). The karyosome is centrally located in the motile form, averaging 2.2μ in diameter and varying from 1.5μ in some forms to 3μ in others. In 2,000 measurements, the nucleus was, on the average, one third the diameter of the organism and the karyosome one half the diameter of the nucleus.

In all stains and especially in iron hematoxylin the karyosome is homogeneous; no centriole, as seen by Nägler in *A. lacertae* (Hartmann) has been observed. Even when the stain has been almost completely extracted from the cytoplasm, the karyosome still remains very dark and never appears either alveolar or vacuolated. In some motile forms the zone surrounding the karyosome is not always clear; in some it is more contracted than in others (Fig. 1) and in such cases the linin threads connecting the karyosome with the nuclear membrane are seen with extreme difficulty.

THE CHROMATINIC GRANULES

There will be noticed a small, black granule (Fig. 3) located near the nuclear membrane. Of the 81 motile forms measured, this cytoplasmic feature was not visible in 13 of them, due to two possible

reasons: (1) to the presence of a large number of metaplasmic inclusions (Fig. 12), or (2) to the cytoplasmic area containing this granule having been cut off in sectioning the intestine. This peculiar structure was observed not only in the motile form but was constantly found in the precystic stage also (Fig. 3). In 889 of these precystic stages only 15 were without this chromatinic granule. At first one may confuse it with the other darkly staining bodies shown in figure 12, but by careful focusing it is seen to have a slightly higher refractive index than the other bodies and is also larger. This granule evidently undergoes division into two parts (Fig. 4); then into four parts (Fig. 5). This process is kept up until the granules become scattered throughout the cytoplasm (Fig. 6). The exact nature of this granule has not been determined, and contrary to Dobell I do not find that it is developed at the expense of the karyosome. At times when food is abundant this deeply staining cytoplasmic body is not to be seen. On the other hand, during periods of starvation it is conspicuous. And with the approach of encystment it divides and becomes a prominent feature of the cell about to become a cyst. These facts suggest that this chromatinic, cytoplasmic inclusion may play a nutritional rôle in tiding the organism through the viscissitudes of starvation and propagation. One may say, therefore, that it represents Meltzer's so-called, "factor of safety."

THE PRECYSTIC FORM

The measurements of 889 of this form show that they are, on the whole, slightly smaller than the motile form; averaging 13.1μ in diameter and ranging in size from 7.5 to 22μ in diameter. They are, in most cases spherical in form so that their measurements are quite easily obtained. The diameter of the nucleus and karyosome is only a trifle smaller than in the motile forms; but this diminution is not sufficient to permit one to decide that the chromatinic granule is formed at the expense of the karyosome. The nucleus averages 4.3μ and the karyosome 2.2μ in diameter, and like the motile form, one finds the nucleus and karyosome increasing or diminishing in size with the individual specimen. The linin threads and "peripheral chromatin" are plainly visible in the precystic stage and especially in the binucleate form (Fig. 13) only four of which were seen in the 2,000 specimens measured. These precysts, like the motile forms are seen all the way from the upper jejunum down to the cloaca, exclusive. No invasion of the tissues by the amoeba in any of its stages has been observed.

THE CYST

This amoeba encysts while still in the small intestine and like the other two forms mentioned above, the cyst is found high up in the jejunum and measurements of 1,030 of them give an average diameter of

12.4μ. The nucleus averages 4.3μ in diameter, while the karyosome in these has an average of 2.2μ. The cysts vary from 7.5 to 22μ in diameter. When compared with both the motile and precystic forms it will be seen that the organism undergoes a reduction of only 2μ in diameter before complete encystment is accomplished. The measurements also show that there is very little variation in the size of the karyosome previous to encystment; the structural diminution seems to be confined chiefly to the cytoplasm and nuclear membrane.

When examined in the living state the cytoplasm appears quite granular and the nucleus very indistinct. The cysts do not react to iodine. In prepared specimens the cyst takes a very deep stain, blue to almost black, but destains more readily than either the precyst or motile form. The karyosome is indistinct (Fig. 9). Previous to encystment all food is voided, the deeply staining chromatinic granules collect near the periphery and the cytoplasm begins to draw away from the cyst wall. This wall is composed of two layers, an outer dark brown layer and an inner yellowish layer (Figs. 7, 8, 9).

No suggestion of a nuclear division has been observed in either the so-called precyst or cyst proper. Only a few of the 81 motile forms studied showed any disposition to divide, so that a complete cycle cannot be given at present. The only suggestion of division observed in the motile form was seen in a case where the chromatin of the karyosome had divided into four granules, two large and two small (Fig. 10); and a second case which was binucleate (Fig. 13).

There was no suggestion of spindle formation in any of these stages such as has been described by Nägler and none was observed by Dobell in the specimens that he studied. It seems very remarkable that in over 100 slides made from fecal smears, and in sectioned intestines of 80 lizards a complete nuclear division was not seen. It may be, however, that division takes place very rapidly in these forms and that one would come upon the division stages by chance. Lack of material prevents further investigation just now.

DISCUSSION

Dobell points out in his report on *A. lacertae,* that there are two amoebae commonly found in the hindgut of *Lacerta muralis;* an Endamoeba (Fig. 15), evidently a parasitic type, and *A. lacertae* (Fig. 14) which is usually accompanied by *Basidiobolus lacertae.* No Endamoeba as illustrated by Dobell has been observed in any of the species of American lizards that have been studied. The basis for assuming that Dobell and Nägler have been dealing with the same species rests upon the nuclear division and not upon the size of the organism. Nägler's report has been so carefully reviewed by Dobell in his own report that it would be a waste of space for me to go over the argument again. The question

now is: Have I been dealing with the same amoeba in *Sceloporus undulatus* that Dobell found in *Lacerta muralis?* In but one respect does it resemble Dobell's amoeba. It resembles his Endamoeba in that division stages could not be definitely established. In seven months of almost continuous investigation only two indefinitely dividing forms, mentioned before, have been observed. Dobell says he worked to the point of exhaustion on his Endamoeba and did not find a single dividing form and for that reason has hesitated to give it a specific name. Our greatest divergence is in the size of the motile form. Dobell's varies from 7 to 14μ in diameter, while mine has a variation of 10.5 to 21μ in diameter. The nucleus of my amoeba varies from 3 to 6μ, his from 3 to 4.5μ. He does not give the size of the karyosome in the motile form but states that it is relatively large. The karyosome in mine varies from 1.5 to 3μ. We agree in that it is homogeneous in character and filled with deeply staining granules; but I have not been able to find them imbedded in a plastic matrix as he has, and in no case does the karyosome ever appear vacuolated.

Dobell finds no "peripheral chromatin" in the clear zone about the karyosome and no linin threads connecting the karyosome with the nuclear membrane. I find a peripheral cloud inside the nuclear membrane and linin threads connecting the karyosome with the nuclear membrane in some specimens, These features are seen only with great difficulty in some specimens and not at all in others. We agree that dividing forms are rounded, but I have seen no pseudopodia and nothing like spindle formation in these forms. In the encystment of the organism we are more nearly agreed than in the comparison made above; Dobell, however, does not describe any transitory stage such as the precystic stage.

A. lacertae, Dobell states, encysts in the hindgut of the lizard. In my investigations cysts and precysts were found high up in the jejunum and ileum and from there on down to the cloaca. I have never seen either a precyst or motile form from the cloaca in *Sceloporus undulatus.* In 889 precysts and 1,030 cysts measured, very little change in the diameter of the karyosome takes place; it is, on the whole, one-half the diameter of the nucleus. As Dobell observed in the cysts of *A. lacertae,* the amoeba dealt with in this paper, gets rid of all food in the precystic stage and becomes spherical; while the linin threads show more plainly in these forms. The origin and purpose of the deeply staining masses, that we both have observed in relation to the nucleus, have not been explained by Dobell. As previously stated, these chromatininc granules apparently originate from a single granule lying near the nuclear membrane, and by successive division become scattered throughout the cytoplasm. It would appear that this substance is used to tide the

organism over the precystic stage and when this transitory period is passed, it is cast out to the periphery of the cytoplasmic wall. About this time the nuclear membrane begins to contract and some of the cysts show some very irregular outlines. I do not find, as Dobell does, that the cysts are hard and impervious to stains; on the contrary, they take such a deep stain and destain so readily that one is able to make out their structure only with great difficulty.

SUMMARY

The amoeba found in the intestine of *Sceloporus undulatus* agrees with *A. lacertae,* as described by Dobell except that:

1. It is larger in the motile, precystic and cystic stages.
2. The nucleus is never vacuolated.
3. A cloud of very fine granules is found inside of the nuclear membrane in motile forms.
4. There is a precystic stage.
5. This organism has a habitat from the upper jejunum down to the cloaca, exclusive.
6. The karyosome averages one half the diameter of the nucleus in the 2,000 forms measured.
7. This organism is characterized in all its precystic stages by a constant chromatinic granule or granules, situated near the nuclear membrane.
8. Division, similar to amitosis, takes place in this granule, until its offspring become scattered throughout the cytoplasm.
9. The cyst stains and destains readily.

From these diverging facts it is evident that this is a new species of amoeba. It apparently belongs to the genus Endolimax; and for the specific name I suggest the name *E. Reynoldsi* as a tribute to the teacher who has been an ever present help and inspiration in my investigations, Dr. Bruce D. Reynolds. I am greatly indebted to Professor William A. Kepner and Dr. James B. Looper for their many courtesies, and particularly to Professor Robert W. Hegner for the perusal of my manuscript.

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Explanation of Plate XXI

The illustrations are drawings and a photograph from sections of the entire small intestine and hindgut of *Scleoporus undulatus* fixed in Bouin's solution and stained with Heidenhain's iron hematoxylin.

Fig. 1.—Motile form showing extended pseudopodium, vacuolated cytoplasm, elongated nucleus and karyosome.

Fig. 2.—Motile form showing polyhedral nucleus and a reticulated cytoplasm.

Fig. 3.—Motile form showing a typical nucleus with central karyosome and linin threads, "peripheral chromatin" and a darkly staining, extranuclear chromatic granule.

Fig. 4.—Early precystic stage showing chromatinic granule dividing.

Fig. 5.—A precystic stage showing chromatinic granule breaking up into four parts.

Fig. 6.—A large precystic stage showing nine chromatinic granules scattered throughout the cytoplasm.

Fig. 7.—Early cystic stage showing light cyst wall and numerous chromatinic granules scattered throughout cyst.

Fig. 8.—Later cystic stage showing chromatinic granules massed around periphery of the cytoplasm.

Fig. 9.—Mature cyst showing chromatinic granules lying at the extreme periphery of cytoplasm.

Fig. 10.—A motile form showing karyosome broken up into four discreet bodies; the chromatinic granule pictured above and many darkly staining bodies of a problematic nature; see text.

Fig. 11.—A motile form showing blunt pseudopodium with a distinction between ecto- and endoplasm; nucleus partially obscured; a single chromatinic granule and many darkly staining bodies.

Fig. 12.—A motile form similar to the last with exception that the darkly staining bodies are more numerous while the nucleus and chromatinic granules are lacking.

Fig. 13.—One of the four binucleate forms encountered. Note the six chromatinic granules three of which are irregular in outline.

Fig. 14.—*A. lacertae* after Dobell, $\times$ 2,000.

Fig. 15.—An "Entamoeba" found in *Lacerta muralis*, after Dobell, $\times$ 2,000.


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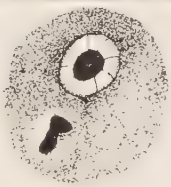
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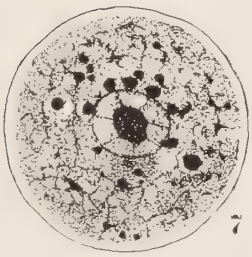
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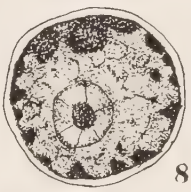
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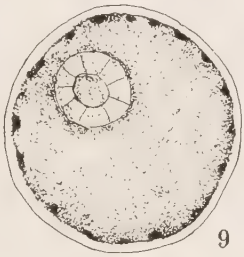
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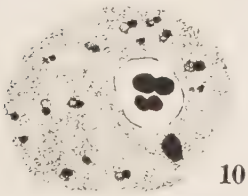
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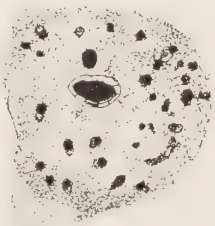
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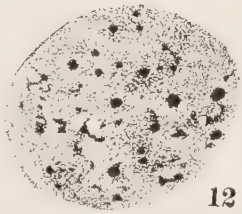
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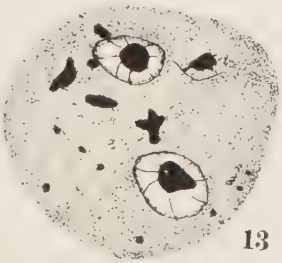
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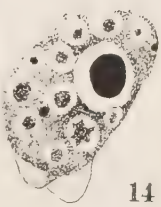
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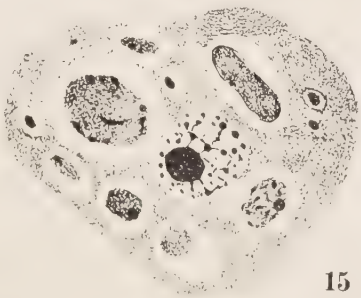
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PLATE XXI

CRITHIDIA ORTHEAE N. SP. FROM REDUVIIDS OF THE GENUS ORTHEA*

CESAR URIBE
Department of Comparative Pathology, Medical School of Harvard University

While looking for the flagellates described by Franchini (1922) and others in the latex of certain species of Euphorbia, some small Reduviidae were found on the leaves and young branches of *Euphorbia pilulifera* which grew profusely in the garden of the Beacon Sun Hospital, Valera, Venezuela, South America. As a matter of routine, the intestinal contents of several of these Reduviidae were examined, and some were found parasitized with a flagellate which appears to be of some morphological interest.

Two different species of Reduviidae were found infected with apparently the same flagellate. The largest of the two, *Orthea bilobata* Say, was found heavily infected in about 10 per cent of the specimens examined; while in the other reduviid, *Orthea vincta* Say, a very slight infection was found in three out of 45 specimens examined. Only infected specimens of *O. bilobata* were used in this study.

The examination of the intestinal contents was carried out in the usual manner, using Locke's solution as a dissecting fluid. This material was examined fresh under coverslips, and films were fixed with osmic acid vapor and stained with Giemsa. A few films were also fixed in Zenker's fluid and stained by the Heidenhain iron hematoxylin method.

In the infected insects the esophagus was always free from flagellates, while the crop or first stomach was heavily infected with numerous large flagellated parasites and other forms which will be described later. Different regions of the intestinal tract showed a variable degree of infection. In almost every case the posterior part of the intestine or second stomach and also the rectum harbored myriads of flagellate and aflagellate forms. In several stained films, leishmania-like bodies were found in the interstices of the epithelial cells and in one instance they seemed to be intracellular, although sections of the intestines of several other infected reduviids did not show these leishmania-like forms in the tissues. The distribution of the different forms was quite irregular. Some types were found indiscriminately in all infected portions of the intestinal tract while others occurred in considerable numbers only in certain definite portions.

The parasite shows varied forms and different sizes which may be accounted for as representing different phases of its development. How-

* Investigations conducted at the Beacon Sun Hospital Laboratory, Valera, Venezuela, S. A.

ever, as the life cycle of flagellates of their type is still under discussion and no definite conclusions have been reached by different workers, it seems advisable to describe all the forms found in the order of their occurrence.

When the crop is ruptured, numerous long, actively swimming forms escape. They vary in size from 15.5 to 50 μ long by 2.5 to 5.5 μ wide and show an elongated body with the flagellar border somewhat twisted and thickened, forming an inconspicuous undulating membrane. The posterior extremity is sharply pointed and in most of the specimens examined, it is slightly curved. The anterior flagellar extremity shows one-half of a complete turn in most of the large specimens; in the small ones it is nearly straight or slightly bent following the curvature of the flagellum.

The protoplasm stained deeply in the anterior portion and shows a very conspicuous vacuole, usually seen anterior to the parabasal body. The posterior portion of the parasite takes a faint stain and shows a few chromidial bodies which are irregularly scattered. These granules are round or ovoid in preparations fixed with osmic acid vapors, but in preparations fixed with alcohol or Zenker, they disappear or are found much distorted and irregularly shaped.

The nucleus has a diameter which is about the same as the width of the parasite in the place of its location. It lies at the junction of the posterior and the middle third of the body, but this situation is quite variable as can be seen in figures 2 and 3. In preparations stained with Heidenhain's hematoxylin, it shows a distinct vesicular type, possessing a large slightly eccentric karyosome. The nucleus is enclosed in a distinct achromatic membrane. The space between the karyosome and the nuclear membrane is free from chromatin. With Giemsa stain, the nucleus is seen as a rather structureless, round mass containing coarse, irregular, chromatic bodies. In some instances, the whole nucleus is surrounded by a clear halo, which seems to be due to retraction of the nuclear substance during fixation.

The flagellum arises at some distance from the parabasal and in most instances, is separated from it by a vacuole, which sometimes encloses its extremity. No basal granule was ever seen, and only in some "resting" stages, connection between the parabasal and the flagellum was observed. From the place where the flagellum arises, it follows one side of the body which is slightly widened so as to form an inconspicuous undulating membrane. The flagellum varies extremely in length and also in proportion to the length of the body. The length of the individual flagellum is found to be from less than half to 15 times the length of the body, varying from 25 to 90 μ (Figs. 3, 5). The flagellum possesses the peculiarity of being unusually thick and may become detached to a variable extent from the margin of the body, probably due to manipula-

tion in preparing the films (Figs. 2, 4, 5, 7 and 11). Small preflagellate and leishmania-like forms are seen attached to the flagellum of the larger individuals as shown in figures 2, 4, 11 and 12. This seems to be due to some property of the small aflagellate forms rather than to any particular stickiness of the flagellum, and the larger aflagellate forms, to be described later on, also often adhere to one another, forming rows of several elements (Figs. 10, 17).

The parabasal body is a rod-like structure usually bent or curved, with its concave side towards the anterior extremity of the flagellate. It is situated anteriorly to the nucleus and at a distance of 2 to 6 μ from it. In dividing and "resting" forms, the distance is greatly reduced. Usually the parabasal lies perpendicular to the flagellum but not infrequently forms with it more or less acute angles. A clear space, having the appearance of a vacuole, is seen immediately anterior to the parabasal (Figs. 1, 2, 3). In dividing forms this vacuole disappears or is very faintly seen, surrounding the parabasal (Figs. 6, 7).

Small leishmania-like bodies were found in association with these elongated forms which represented the larger percentage of the parasites in the crop. These are much smaller than the so-called "resting" forms with coiled flagellum and somewhat smaller than the "postflagellate" forms to be discussed further on. They are round or ovoid bodies, measuring about 3.5 μ in their greatest diameter. Numerous forms are seen attached to each other (Figs. 10, 17) and to the flagellum of the larger parasites. Elongated aflagellate organisms with pointed extremities were also found in the crop, but more abundantly in the second stomach or gut, where they represented the largest percentage of the forms found (Fig. 11). These forms are very small, measuring about 10 μ in length and so slender that finer structural details were not distinguishable.

The posterior part of the gut, especially the rectum, contained another type of flagellate which is very much like an Herpetomonas. The organisms of this type showed a narrow, elongated body and the parabasal so near the anterior extremity, that it was impossible to demonstrate an undulating membrane (Fig. 11). The so-called "resting" forms are rounded often oval organisms measuring from 6 to 10 μ with the flagellum wound spirally around the body. These together with the small "postflagellate" forms were distributed throughout the infected portion of the intestinal tract, both types being most numerous in the rectum. The "postflagellate" forms are considerably smaller, having a diameter of 4 to 6 μ, and are commonly seen in rows of 5 or more elements (Fig. 10). The parabasal and the nucleus, which are much reduced in size, lie close to each other and are often seen against the body wall.

In all the stained preparations numerous dividing forms were found, showing different stages corresponding to the well known process of

longitudinal binary division seen in similar flagellates. No rosette or multiple division, or the endogenous budding described by McCulloc (1919) for *Crithidia euryophthalmi* was found. The inoculation of N.N.N. medium and injection into young white rats and guinea-pigs gave negative results.

Descriptions of Crithidia as found in the available literature do not correspond with the type described in this paper. The name of *Crithidia ortheae* is here proposed for it.

SUMMARY

Two reduviids, *Orthea bilobata* Say and *O. vincta* Say, found on leaves and branches of *Euphorbia pilulifera* were found parasitized with flagellates. Of the 200 specimens examined, 10 per cent of *O. bilobata* were parasitized. This infection was found to be almost continuous from the crop to the rectum.

Various types of organisms are described which are interpreted as developmental forms of a single species.

Forms occur showing a very long flagellum (15 times the length of the body). Elongated forms without flagella were found in the middle and posterior gut, and slender, active forms were found in the region of the rectum. There were also present minute leishmania-like organisms, large rounded forms with coiled flagellum and "postflagellate" forms which showed a marked tendency to adhere to one another in rows.

Animal inoculations and cultures were negative.

The name of *Crithidia ortheae* is proposed for this species.

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EXPLANATION OF PLATE XXII

Figs. 1, 2, 3.—Large types of flagellated crithidia from the crop. In figure 2 the flagellum is detached from border of body.

Fig. 4.—Binucleated form. (Small leishmania-like forms adhering to the flagellum of large flagellates shown in figures 2 and 4.)

Fig. 5.—Small form with extremely long flagellum, also detached from border of anterior extremity of body.

Figs. 6, 7.—Dividing form.

Figs. 8, 9.—"Resting" forms; figure 8 showing coiled flagellum.

Fig. 10.—"Postflagellate" forms.

PLATE XXII

Explanation of Plate XXIII

Fig. 11.—Showing types of flagellates and aflagellate elements occurring in the crop; *a*, Leishmania-like forms appearing to be included within a cell; *b*, Small herpetomonas; *c*, Large herpetomonas types; *d*, Elongated aflagellate forms; *e*, Large crithidia.

Fig. 12.—Clump of flagellates from the crop.

Fig. 13.—Conglomerate of "postflagellate" forms.

Figs. 14, 15, 16, 17, 18.—Diagrammatic representation of all the forms found.

PLATE XXIII

GIARDIA BECKERI N. SP. FROM THE GROUND SQUIRREL AND *ENDAMOEBA DIPODOMYSI* N. SP. FROM THE KANGAROO RAT*

R. W. Hegner

One of the most interesting phases of the study of host-parasite relationships is host-parasite specificity, and some of the best material for the investigation of this subject is to be found in the genus Giardia. Giardias were first described from man by Leeuwenhoek in 1681 (Dobell, 1920). The genus name, Giardia, was first applied to the species in the tadpole, *Giardia agilis,* by Künstler (1822). Giardias were later found in mice, rats, cats and rabbits as well as in man by a number of investigators but no species differences were recognized until 1908 when Bensen distinguished three species, *G.* (*cuniculi*) *duodenalis* from the rabbit, *G. muris* from the mouse, and *G.* (*intestinalis*) *lamblia* from man. To this list have been added *G. microti* from meadow mice, *G. canis* from dogs, *G. caviae* from the guinea-pig, *G. simoni* from rats and *G. cati* from cats. Other giardias have been described from reptiles and birds. The genus exhibits remarkable host-parasite specificity since almost every species of mammalian host appears to be parasitized by its own peculiar species of Giardia. The principal morphological characteristics used to distinguish these various species are length, breadth, ratio of length to breadth, distance from the anterior end of the body to the center of the nucleus, from the center of the nucleus to the end of the lateral shields, from the end of the lateral shields to the posterior end of the body; distance across the body at the center of the nuclei and at the ends of the lateral shields; the contour of the body, whether narrow or broad at the anterior end and across the lateral shields; distance of nuclei from the median line and from the posterior edge of the sucking disc; size and shape of the nuclei; number, size, shape and location of the parabasal bodies; and staining characteristics.

Recently Dr. Elery R. Becker sent me several slides, fixed in Schaudinn's solution and stained with iron-hematoxylin, which contain giardias obtained from two of seven ground squirrels, *Citellus tridecemlineatus* Mitchill, captured at Ames, Iowa. These giardias differ morphologically from any heretofore described and I, therefore, propose for them the specific name *beckeri* after their discoverer.

A camera-lucida drawing of this new species is shown in figure 1; a diagram of a specimen magnified 2800 diameters so that it can be com-

* From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

pared with other mammalian species (see Hegner and Taliaferro, 1924: p. 246) is presented in figure 2; and a diagram of *G. microti* is shown in figure 3, thus making it possible to contrast the characteristics of two species that occur in hosts (ground squirrel and meadow mouse) that live in the same general habitat. *G. beckeri* resembles *G. duodenalis* from the rabbit more than any other species. The sides of the body slope rapidly from the point of emergence of the antero-lateral flagella to the anterior end, and the breadth across the lateral shields is comparatively greater than in most of the other species. This characteristic gives the animal a pinched in appearance across the body at the ends of the lateral shields. *G. beckeri* is, however, much smaller than the species from the rabbit, averaging only 13.7μ in length and 7.2μ in breadth; whereas *G. duodenalis* averages 15μ in length and 8.9μ in breadth. The guinea-pig giardia, *G. caviae,* which *G. beckeri* also resembles is much shorter than the latter, averaging only 10.7μ in length although these two species are of practically the same breadth.

Giardias no doubt reach their entozoic habitat in the duodenum of their hosts in the cyst stage by way of the mouth when contaminated food is eaten. The species, therefore, that would be most liable to find its way into the digestive tract of the ground squirrel would be *G. microti* of the meadow mouse. The measurements of ten specimens of *G. beckeri* taken at random are for this reason contrasted with similar measurements published by Simon (1922) of *G. microti.*

	G. beckeri Microns	G. microti Microns
Length of body	13.68	11.06
Breadth of body	7.19	6.79
Anterior end to center of nucleus	3.94	3.37
Center nucleus to end of lateral shields	5.05	4.68
End of lateral shields to posterior end	4.99	3.87
Breadth across end of lateral shields	3.71	3.80
Breadth across center of lateral shields	7.04	6.21*

* From slides made by Dr. Simon.

These measurements show *G. beckeri* to be both longer and broader than *G. microti.* The breadth of the former across the center of the lateral shields is considerably greater than that of *G. microti* but is more conspicuous when the animal itself is examined than would appear from the figures. The range in length and breadth and averages of these dimensions for one hundred specimens of *G. beckeri* taken at random are as follows:

Length: shortest, 8.8; longest, 17.0; average, 13.68μ.

Breadth: narrowest, 5.2; broadest, 9.6; average 7.19μ.

The parabasal bodies of *G. beckeri* are two in number, comma-like in shape, and extend from one-third to one-half the distance across the body just posterior to the sucking disc.

ENDAMOEBA DIPODOMYSI N. SP.

The endamoebae resemble the giardias in their rigid host specificity. Species that are apparently distinct have been described from man, monkeys, mice, rats, guinea-pigs and others mammals as well as from other classes of vertebrates and from invertebrates. As a rule, each host in nature seems to be parasitized by its own particular species and usually cross infection appears only under experimental laboratory conditions. A new species of Endamoeba was recently found in this laboratory in the kangaroo rat, *Dipodomys spectabilis* Merriam, obtained by purchase from New Mexico. This Endamoeba consisted mostly of granular endoplasm and moved by the sudden thrusting out of small hyaline pseudopodia, resembling in this respect, *E. histolytica* from man. No measurements were made of the living organisms. When fixed in Schaudinn's solution and stained with iron-hematoxylin no clear ectoplasm is visible. The body is rather broadly oval in shape as shown in figure 4. Specimens range in size from 12.1 to 16.3μ in length with an average length of 14.1μ and from 10.1 to 13.9μ in breadth with an average breadth of 12.2μ. There is nothing distinctive about the cytoplasm. The nucleus is comparatively large, ranging from 3.48 to 4.79μ in diameter with an average diameter of 4.19μ. It is distinctly of the endamoeba type with one large karyosome and a peripheral layer of chromatin granules. The karyosome is eccentrically located and often irregular in outline as though made up of a number of granules. The peripheral chromatin consists of granules arranged in a layer on the inside of the nuclear membrane. These granules are so often found to be smaller on the side of the nucleus nearest the karyosome that this characteristic may be of some significance. No chromatin granules occur between the karyosome and the nuclear membrane. It is quite different from *Endamoeba ratti* and *Councilmania muris* and *C. decumani* as described by Kessel (1924). No cysts of *E. dipodomysi* were found. For this new species of Endamoeba I propose the name *dipodomysi* after the species of kangaroo rat in which it was found.

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EXPLANATION OF PLATE XXIV

Fig. 1.—*Giardia beckeri* n. sp. Camera lucida drawing (× 4000) of a typical specimen.

Fig. 2.—*Giardia beckeri* n. sp. Diagram of a specimen showing average measurements (× 5600).

Fig. 3.—*Giardia microti.* Diagram of a specimen showing average measurements (× 5600).

Fig. 4.—*Endamoeba dipodomysi* n. sp. Camera lucida drawing (× 4000) of a typical specimen.

Fig. 5.—*Endamoeba dipodomysi.* Outline drawings (× 2300) showing variations exhibited by different specimens.

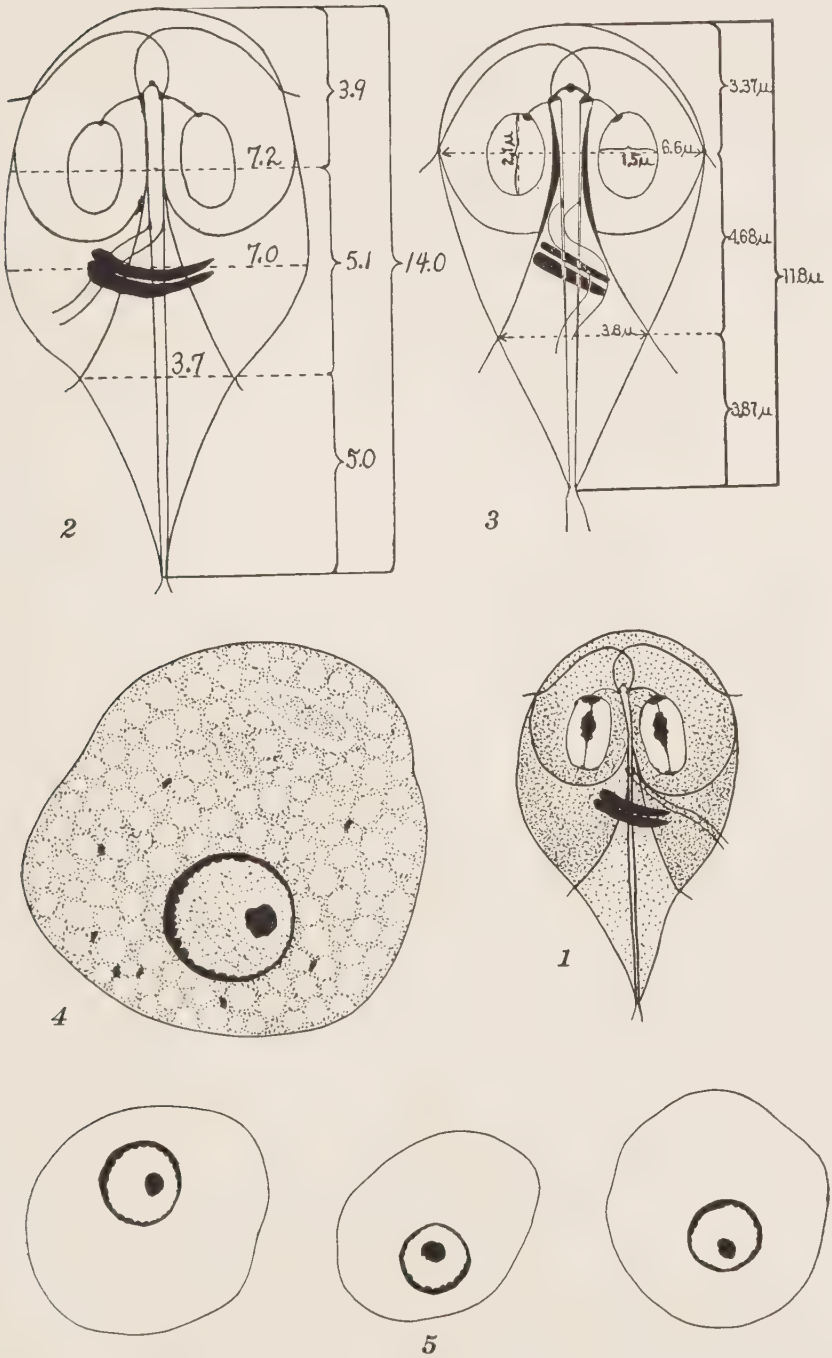


PLATE XXIV

A TREMATODE WITH TWO ANI*

George R. La Rue

Ozaki (1925) has recently described two species of trematodes with a true anus which opens directly to the exterior on the ventral surface a short distance in front of the posterior end. His review of the literature on the subject contained references to three papers (Leiper, 1908; Odhner, 1910, 1911), which discussed species in which the intestinal ceca discharge into the excretory vesicle. The specimens observed by me present a condition different from any of the above. The intestine is bifurcated and each intestinal fork opens separately by means of a narrow duct, situated one on either side of the excretory pore.

These specimens were first described by MacCallum (1918) under the name of *Hemistomum haustrum,* host *Alutera schoepfii,* from the southeast coast of the United States. Since Hemistomum Diesing is a synonym of Alaria Schrank and since these specimens are not congeneric with *Alaria alata* (Goeze), the type of Alaria, and cannot in fact be placed in any existing genus of the family Strigeidae and probably do not belong in that family, I propose for them the new generic name Diploproctodaeum with *Hemistomum haustrum* Mac Callum as the type species.

Diploproctodaeum haustrum (MacCallum)

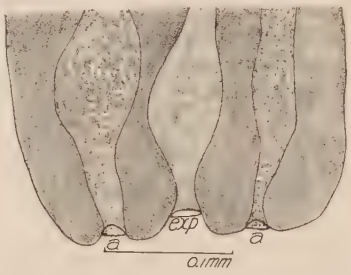
The material of this species which Dr. MacCallum has kindly permitted me to study, consists of seven specimens mounted *in toto* on one slide. One of these specimens is clearly the one portrayed in MacCallum's figure 47. As described and figured by him these worms have an arrangement of sexual organs typical for distomes while the body is divided into two body regions, the anterior part being spoon- or scoop-shaped and the posterior cylindrical. In form of body this species resembles species of Neodiplostomum Railliet. An examination of the specimens with a 4 mm. dry objective reveals the details of structure as presented in MacCallum's description, but if his excellent preparation is studied with a 3 mm. oil immersion lens some additional features are clearly revealed.

The entire forebody is richly glandular, the gland cells being most abundant near the union of the two body regions. In this respect this species resembles Neodiplostomum but since sections are not available for study it is impossible to make a detailed comparison. The cuticula

* Contribution from the Zoological Laboratory of the University of Michigan.

is spinous but it is not possible to determine the distribution of spines with any precision. Large spines arranged in the form of a collar about the anterior end appear to be wholly lacking, nor is there any appearance of a collar which has lost its spines. There is no holdfast organ and no visible trace of an adhesive gland. The acetabulum is large, with a weak musculature, and a well marked cavity.

The intestinal branches open separately at the posterior end of the body through narrow ducts, situated one on either side of the excretory pore (Fig. 1). Although the ani are not visible in all the specimens, there can be no doubt that they are normal structures, since there is no evidence that the posterior end of the worm has been broken off. The intestine is filled with masses of epithelial cells of host origin, an indication that this parasite may cause considerable damage to its host. The reproductive organs are arranged just as MacCallum figured them except that I have been unable clearly to see Mehlis' gland. With the 3 mm.



Posterior end of *Diploproctodaeum haustrum* (McCallum) showing ani and excretory pore. Masses of fecal material shown in intestine.

oil immersion lens it can be determined that the swollen terminal portion of the male duct is a cirrus pouch containing a cirrus. In a single specimen the cirrus is protruded.

Generic diagnosis.—Distome. Body divided into two regions, anterior spoon- or scoop-shaped, posterior cylindrical. Forebody glandular. Cuticula spinous. Oral sucker sub-terminal, acetabulum large. Genital pore anterior to the acetabulum. Cirrus and cirrus pouch present. Testes in series in hind body. Ovary anterior to testes and to the right. Oötype apparently anterior to testes. Vitellaria in hind body. Pharynx large. Intestine bifurcate, each fork opening at posterior end through a separate anus. Parasitic in intestine of fish.

Relationships.—In external appearance *Diploproctodaeum haustrum* resembles species of Neodiplostonum Railliet and at the same time it resembles Chaunocephalus Dietz and Scapanosoma Lühe but lacks the large spines characteristic of these genera. In arrangement of the

reproductive organs it resembles the latter two species much more than Neodiplostomum. In the absence of any information as to the life history of this species it is impossible to determine with certainty its relationships. For that reason no attempt has been made to place it in a family.

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SCYPHIDIA CLYMENELLAE, N. SP., AN ENDOPARASITIC PERITRICH

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Scyphidia clymenellae n. sp.

While studying the life history of *Haplozoon clymenellae,* my attention was directed to a ciliated protozoan frequently found in the posterior end of the alimentary tract of *Clymenella torquata,* the annelid parasitized by Haplozoon. A more detailed study of the ciliate revealed the fact that it is an undescribed species of the vorticellid genus, Scyphidia. Several of the species of this genus are described as ectoparasites, chiefly on freshwater naids, but the individuals here recorded are unique in their endoparasitic habitat, and represent a step in parasitism more advanced than that of any of their relatives.

Diagnosis.—Body elongate and conical, length 100μ extended, ratio of body length to diameter of peristome when extended 3:1; highly contractile; free-swimming or attached by a narrow scopula at the end of a short contractile epistyle; usually solitary, sometimes temporary associations of two or four individuals formed by incomplete division of the epistyle. Peristome large; disk prominent; single contractile vacuole at distal end of the body, macronucleus large and ribbon-like, micronucleus large and oval, located in the disk. Taken in the posterior end of the intestine of *Clymenella torquata* at Woods Hole, Mass. Most resembles *S. terebellae,* Fauré-Fremiet, from which it differs in shape (ratio of body length to peristome diameter lower), absence of a projection on the disk, and habitat (text fig. 1).

The genus Scyphidia, Dujardin, was established to receive the type species *S. rugosa,* a fresh-water form which greatly resembles the species here described except for a lower ratio of body length to peristome diameter (2:1) and the presence of annular ridges. Lachmann later excluded this type species and others described by Dujardin, regarding them as well as the species included by Fromentel, Perty and others as immature Vorticellae, but retained the generic name for two new species, *physarum* and *limacina,* characterized by cylindrical bodies, the absence of an epistyle, and a small peristome. Both species were taken attached to the naked parts of the molluscs Physa and Planorbis. The species added since the time of Lachmann resemble more nearly the original type of Dujardin. Most of them are fresh-water forms,

* Contributions from the Zoological Laboratory of the University of Illinois, No. 277.

attached often to naids such as *inclinans* D'Udekem, *constricta* Stokes, or *ovata* Kellicott. Two marine species, both attached to annelids are recorded, *S. scorpaenae* Fabre-Domergue, and *terebellae* Fauré-Fremiet.

Stained preparations demonstrate the nature of the attaching organ or scopula to be identical with that described by Fauré-Fremiet. At the end of the short elastic stalk or epistyle there is a shallow inverted cup containing modified cilia by means of which the animal attaches itself to the gut-epithelium of the host. The attachment is easily broken off and as easily a new attachment made. The stalk consists of an outer vase-like mass of longitudinal contractile fibers, while the cytoplasm of the body continues into the cavity of the vase (text fig. 2).

The habitat of this species is the posterior end of the intestine of *Clymenella torquata*. Contrary to expectations, no scyphidia were found

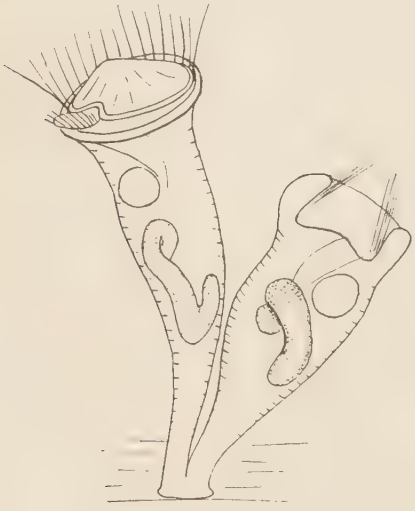

Figure 1.

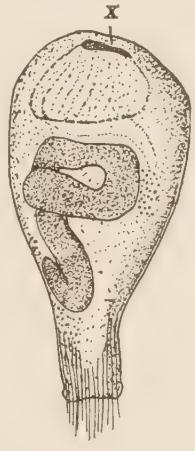

Figure 2.

Text Figure 1.—Two individuals redrawn from a camera sketch from life. × 100.

Text Figure 2.—Camera-sketch from a specimen fixed in sublimate-acetic and stained in iron-hematoxylin (× 120). X indicates position of micronucleus.

attached to the cuticula of the worms. The percentage of infection is high, approximately 50%. Since my own observations on these parasites in 1919 and 1922, I am informed by Prof. D. B. Young that they have been frequently encountered in Clymenellae used as laboratory material in the invertebrate course at the Marine Biological Laboratory.

Many of the species of this genus are attached to the integument of fresh-water and marine annelids. *S. clymenellae*, however, is the first to be discovered inside the body of the host. Its restriction to the posterior

end of the intestine leads to the conclusion that its entry is made by way of the anus and that the periodic dilution of this region of the intestine by fresh sea-water permits the protozoan to effect a lodging here. It is an interesting fact that within this genus are contained non-parasitic species such as *S. rugosa,* ectoparasites such as *S. terebellae,* and endoparasites as represented by *S. clymenellae.* So far as I have been able to discover, this species is the first endoparasitic peritrich to be recorded.

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A NEW INVERTEBRATE HOST OF *TRYPANOSOMA CRUZI* CHAGAS *

Cesar Uribe

Among some specimens of *Rhodnius prolixus* Stahl received at the Beacon Sun Laboratory, Valera, Venezuela, S. A., from the town of Trujillo, Venezuela, were found one living and one dead specimen of a black Reduviid bug, later on classified as a female adult of *Apiomerus pilipes* Fabr. All the insects were claimed to have been collected from a single shack in the outskirts of the above mentioned town. There were in all forty-three larvae, nymphae and adults of *Rhodnius prolixus,* which were separated into three lots and the living specimen of *A. pilipes* was placed with one of these lots containing several larvae and nymphae full of blood.

On the following day, half of the insects kept with *A. pilipes* were dead and on close examination of these, a small hole in one of the interchitinous membranes between the dorsal segments of the abdomen was found in each. The abdomen of some of these dead insects was collapsed, evidently showing that the bloody contents with which they were distended was sucked through the hole made in their backs. *A. pilipes* was suspected of being the cause of the mortality in the Rhodnius stock, so it was placed in a separate bottle. On examination of the intestinal contents of the dead bodies of Rhodnius, an unusually rich infection with *Trypanosoma cruzi* was found in each.

The feces of *A. pilipes* were examined during the ten following days but no flagellates were seen. During all this time it refused to suck blood from newly born white rats or from the human finger which was presented several times during day and night.

After this period of fasting, a small half starved third stage larva of *R. prolixus* was put into the bottle with *A. pilipes.* The latter immediately went after the Rhodnius larva and seizing it with its two anterior legs, inserted its powerful proboscis through the soft integument of the articulation of the head and thorax, paralyzing the victim and sucking from it for a period of about six minutes, after which the dead larva was dropped. Three more thin and starved larva were put in the bottle and one after another were subjected to the same process as described for the first one.

One fully distended larva was dropped in the bottle and immediately was seized by the voracious *A. pilipes* which held it fast with its powerful legs and by means of its proboscis pierced the soft membrane between

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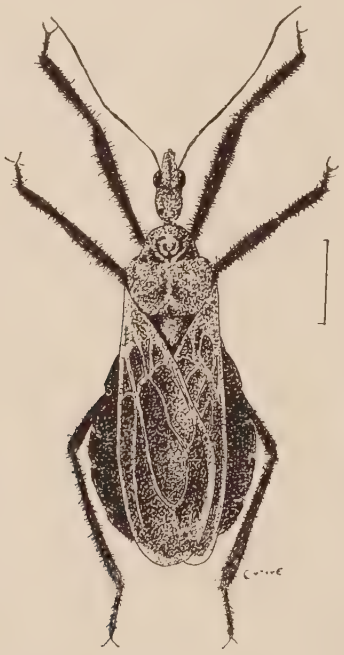
the dorsal chitinous segments. The intestinal contents of the larva were evidently sucked out, because after being fed upon for nearly ten minutes, its abdomen was much reduced in size.

During a period of four days, five more fully distended nymphae were put in the bottle containing *A. pilipes,* and all of them were sucked nearly empty through a hole pierced in the dorsal side of the abdomen. The bodies of these nymphae were examined and in four of them, numerous forms of *T. cruzi* were found in the intestinal contents.

From the tenth day after the first killing of Rhodnius, the Reduviid was kept without food for five days and daily examination of its feces



was made, but no flagellates of any description were found. At the end of this period two common house flies, placed in the bottle with *A. pilipes,* were killed by the latter which fed upon them in the same way as it did upon starving Rhodnius larvae, by piercing the membrane of the articulation of the head and the thorax. The intestinal contents of each of these two flies was examined and found negative as to the presence of flagellates.

Seventeen days after the female *A. pilipes* was received in the laboratory, it was found dead in the bottle without visible explanation of the cause of its death. The body was opened through the ventral side of the abdomen and the intestines taken out. They were found full of a blackish sticky fluid resembling partially digested blood and when

examined under the microscope, myriads of flagellates were seen swarming throughout the entire length of the intestinal tract. Smears stained with Giemsa showed innumerable flagellates morphologically identical with the different forms of *T. cruzi* found in *Rhodnius prolixus.*

Three dark operculated eggs, apparently mature, were taken out of the insect and incubated for several days but none of them ever hatched, so nothing was learned of its life history.

Through the courtesy of Prof. J. M. Aldrich of the Smithsonian Institution, this insect was identified as *Apiomerus pilipes* Fabr.

SUMMARY

An adult female *Apiomerus pilipes* Fabr. was found in association with *Rhodnius prolixus* in a shack near Trujillo, Venezuela, S. A. This Reduviid, which fed readily upon *Rhodnius prolixus* Stahl and also upon *Musca domestica* Linn., refused to suck blood from newly born white rats, and also from man.

Dying after several feedings upon body juices and apparently upon the intestinal contents of *Rhodnius prolixus* which contained *Trypanosoma cruzi,* its intestinal contents showed a very heavy infection with flagellates morphologically identical with the forms of *T. cruzi* found in *R. prolixus.* Daily examination of its feces had not shown flagellates of any description.

While little was learned of the biology of *A. pilipes,* it is clearly indicated that it feeds upon *Rhodnius prolixus,* with which it was found associated, rather than upon vertebrates, and that it may serve as a host of *Trypanosoma cruzi.*

A NEW HUMAN TREMATODE, *HETEROPHYES KATSURADAI* N. SP.

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The worms which form the subject of this paper were obtained by anthelmintic means from a man suffering from diarrhea in the Settsu Hospital at Kobe, Japan. We wish to thank Dr. Fujiro Katsurada the director of the hospital, for giving us the opportunity to study them, and we dedicate this new species to him who has done so much for the study of human trematodes in Japan. To Prof. S. Goto we express our most hearty appreciation of the unfailing interest, which he has taken in our work, and the many kindnesses received.

The general outline of the worm is broadly oval or cone shaped, with pointed cephalic pole and transversely rounded caudal end. Length 0.61 to 0.89 mm.; greatest breadth, which occurs a little behind the middle of the body, 0.40 to 0.47 mm.; it is therefore a small species. The body is covered with small simple spines. The oral sucker is globular, 61 to 63μ in diameter. The acetabulum is situated at about the junction of the cephalic and the middle thirds of the body length, with rounded outline and triangular aperture in most specimens; diameter varying from 195 to 220μ therefore greater than a quarter of the body length. Neck, prepharynx and esophagus very short, prepharynx 11 to 12μ long, esophagus 24 to 25μ, pharynx measuring 45 by 35μ. The intestinal bifurcation takes place midway between the pharynx and the anterior border of the acetabulum. The right cecum runs backward parallel to the edge of the body, and ends at the central level of the right testis on its outer side, never extending farther. The left cecum ends symmetrically with the right in six out of the 19 specimens (32%), and in 13 (68%) extends to the posterior end of the body beyond the right testis. The genital sucker is elongated, with a slight depression on the side next the acetabulum, and lies obliquely to the left of and behind the latter. It measures 0.11 to 0.14 mm. in length and 0.07 to 0.085 mm. in breadth; the crown of chitinous rodlets interrupted on the side next the acetabulum; rodlets slightly curved, each with four pointed lateral processes, 52 to 57 in number.

The testes are situated at the posterior end of the body, with the right one displaced slightly behind the left. They are globular in shape, 0.08 to 0.14 mm. in diameter. The seminal vesicle is well developed, and lies behind the acetabulum near the dorsal surface. It is comparatively short and curved, measuring about 0.09 by 0.06 mm. There is a globular pars prostatica with prostatic cells. The ductus ejaculatorius is fairly long but almost straight.

The ovary is globular and situated in the median line midway between the acetabulum and the testes, i. e., at the junction of the second and the last thirds of the body. It lies on the ventral side and measures 55 to 92μ in diameter. From its dorsal surface the oviduct arises and passes forward and soon turns posteriad. A small shell gland complex lies on the antero-dorsal side of the ovary, and a large receptaculum seminis (0.11 by 0.06 mm.) to the left of and behind the ovary near the dorsal surface. The uterus passes caudad from the shell glands on the right side of the median line, and describing a complex loop on the left anterior side of the left testis passes again to the right side of the initial portion, where it describes dense convolutions and passes on to the left side of the body and after several convolutions again crosses over to the right side, just behind the acetabulum, and after some additional convolutions proceeds to the genital aperture. The vitellaria are fairly developed; laterally they extend from the level of the anterior border of the ovary to the central level of the testes; on the dorsal side they are seen to cross the median line and unite in front of the testes. The lobules are about 8 to 14 on each side and 14 to 20 in the transverse connecting part. The uterine eggs are very numerous and measure 25.3 to 25.9μ by 14.3 to 15μ; they are yellowish brown, and the shell is thickened to form a slight knob at the abopercular pole. Miracidia are already formed in the uterus.

Heterophyes katsuradai differs from the other species of this genus in the relative size of the acetabulum to the body length, the shortness of the neck, and the distribution of the vitellaria. The acetabulum is remarkably larger than in any other species relatively to the body, its diameter being greater than a quarter of the body length. The vitellaria of the two sides cross the dorsal median line to unite with each other at the anterior border of the testes. On the basis of these characteristics, we propose a new species with the following diagnosis.

Heterophyes katsuradai n. sp.

Length 0.61 to 0.89 mm.; maximum width 0.40 to 0.47 mm., short oval, anterior end pointed, posterior end obtuse. Cuticula with spines. Oral sucker globular, 61 to 63μ in diameter; acetabulum very large 195 to 220μ in diameter, on the boundary between the cephalic and middle thirds of the body. Genital sucker on the left side of and behind the acetabulum, 0.11 to 0.14 mm. by 0.07 to 0.085 mm. Chitinous rodlets of the genital sucker 52 to 57 in number, each with four pointed lateral processes directed towards the free end of the rodlet. Intestinal cecum of the right side always ending at the central level of the right testis, that of the left frequently extending beyond the left testis. Testes globular, right testis somewhat more posterior than left, 0.08 to 0.14 mm. in diameter. Seminal vesicle behind the acetabulum under the dorsal surface. Ovary 55 to 92μ in diameter, in the median line in front of testes. Receptaculum seminis on the left side of and behind the ovary, about 0.11 by 0.06 mm.; uterine coils filling up spaces between testes and acetabulum. Vitellaria extending from the level of the anterior border of the ovary to the central level of the testes,

those of the two sides coalescing at the anterior border of the testes. Eggs thick shelled, yellowish brown, 25.3 to 25.9μ long, 14.3 to 15μ wide. Miracidium ciliated, developed in the uterus.

Habitat: Intestine of man.

Locality: Kobe, Japan.

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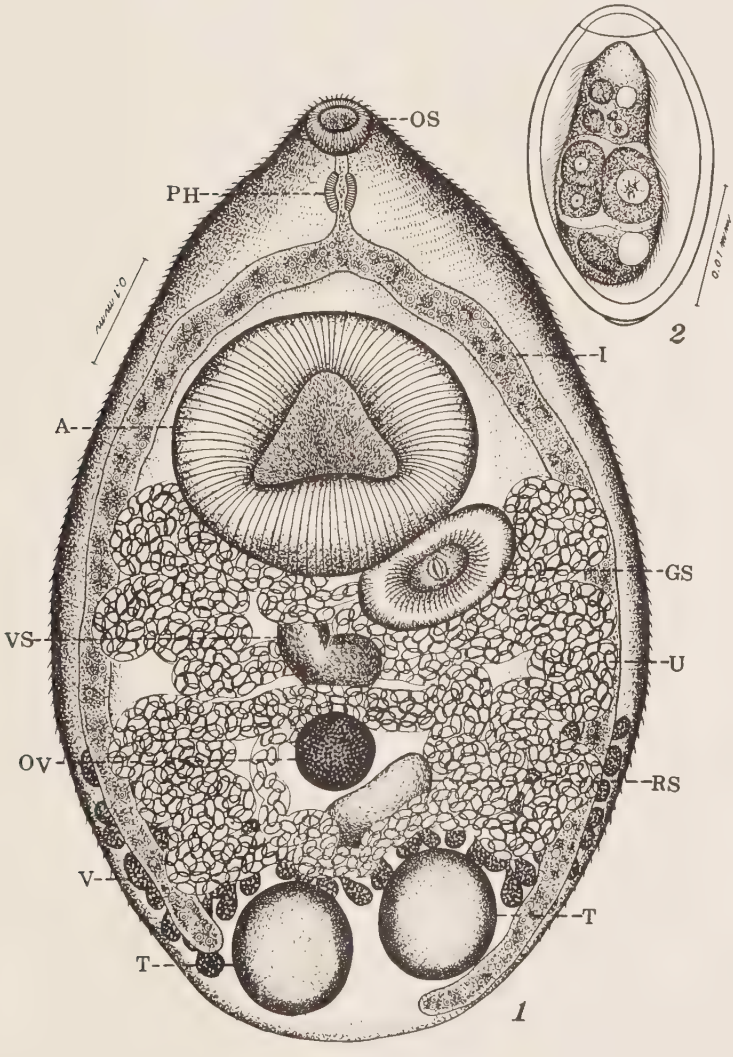

PLATE XXV

EXPLANATION OF PLATE XXV

Heterophyes katsuradai

Fig. 1.—Ventral view.

Fig. 2.—Egg.

A Acetabulum
GS Genital sucker
I Intestine
OS Oral sucker
OV Ovary
PH Pharynx
RS Receptaculum seminis
T Testis
U Uterus
V Vitellaria
VS Vesicula seminalis

DETECTION OF INTESTINAL PROTOZOAN INFECTIONS BY THE CULTIVATION METHOD

Elery R. Becker

Iowa State College

The diagnosis of intestinal flagellates by culture methods was advocated by Hegner and Becker (1922) in a previous paper. Those writers made fecal examinations of 110 individuals by the smear and culture methods. In two cases, intestinal flagellates were uncovered by the smear method. The culture method revealed eight infections. Similarly, flagellates could be cultivated from stools known to contain *Chilomastix mesnili* and *Trichomonas hominis* after so many of the flagellates contained in the stool had undergone degenerative changes that they could no longer be found in smears from these stools. The flagellates cultivated were *Chilomastix mesnili, Trichomonas hominis,* and a smaller one which in unstained preparations resembled the descriptions of *Enteromonas hominis.* Stained smears of the latter flagellate were not sufficiently clear to confirm the diagnosis made of the living forms in the culture. The medium employed was the ovomucoid, which had previously been employed by Hogue (1921) for the cultivation of *Embadomonas* (*Waskia*) *intestinalis* and *Trichomonas hominis.* Reichenow (1923) using a different medium confirmed the essential points of Hegner and Becker's work, except that he found Chilomastix somewhat more capricious in its cultivation.

All the valid species of intestinal flagellates of man, with the exception of *Giardia lamblia,* have now been cultivated. Lynch (1915), and later Ohira and Noguchi (1917), deserve credit for having first cultivated *Trichomonas hominis.* Lynch used boullion slightly acidified with acetic acid. Boeck (1921) cultivated *Chilomastix mesnili* on a medium consisting of one part human blood serum and four parts Locke's solution. *Embadomonas* (*Waskia*) *intestinalis* was first cultivated by Hogue (1921) on the ovomucoid medium, which is the filtrate of the heated mixture of the white of hen's eggs and 0.7 per cent salt solution. Hegner and Becker (1922) grew from the feces of two hospital patients flagellates which answered the description of *Enteromonas hominis.* Boeck (1924) was able to cultivate *Tricercomonas intestinalis* on his Locke-egg-serum medium. Hogue's ovomucoid medium will grow *Trichomonas hominis, Chilomastix mesnili, Embadomonas intestinalis,* and *Enteromonas hominis.* It remains to be seen whether *Tricercomonas intestinalis* will grow in it. At any rate, so far as is known, the best results in the diagnosis of intestinal flagellate infections may be expected from the use of the ovomucoid medium.

Through the kindness of Dr. Cotton, who is in charge of the New Jersey State Hospital at Trenton, the writer was given the opportunity of making fecal examinations of 103 patients at this hospital. Both smear and culture diagnosis methods were employed. Two smears were made from each stool, one in normal saline and one in diluted Lugol's solution. The culture technic was the same as that employed by Hegner and Becker in their previous work on intestinal flagellates. The smear diagnosis of the 103 stools yielded the following protozoan infections:

Endamoeba coli (cysts)	38
Endolimax nana (cysts)	2
Endamoeba histolytica (cysts)	2
Iodamoeba williamsi (cysts)	1
Giardia lamblia (cysts)	2
Chilomastix mesnili (cysts)	5
Trichomonas hominis (motile)	2

It can be seen at a glance that outside of the abnormally high percentage of infections with *Endamoeba coli,* there is nothing extraordinary in the above data.

Trichomonas was grown in cultures from three of the stools, the two in which it had been detected by the smear method, and one other. *Chilomastix* was grown in culture from only two of the above five stools in which it had been detected by the smear method. Since the material was not received until the day after it was mailed, it is not surprising that it was not cultivated from the other three cases. Becker and Hegner (1922) showed that in the case of one stool, *Chilomastix mesnili* could not be cultivated from it more than eighteen hours after the stool was passed.

SUMMARY

Faecal examinations of insane patients in the New Jersey State Hospital by the smear method uncovered two cases of *Trichomonas hominis.* The culture method disclosed these two, and one more.

Chilomastix mesnili was encountered in five stools by the smear method. It appeared however, in only two cultures. This poor showing was probably due to the fact that the material was about a day old when received.

The culture method shows distinct advantages over the smear method in the detection of *Trichomonas hominis* in feces, but for the detection of *Chilomastix mesnili* it should be used in connection with the smear method.

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Hegner, R. W., and Becker, E. R. 1922.—The diagnosis of intestinal flagellates by culture methods. Journ. Parasit., 9: 15-23.

Hogue, M. J. 1921.—The cultivation of *Trichomonas hominis.* Am. Jour. Trop. Med., 1: 211-214.

BOOK REVIEWS

THE NEMATODE PARASITES OF VERTEBRATES. By WARRINGTON YORKE and P. A. MAPLESTONE. 536 pp., 307 figs. P. Blakiston's Son & Co., Philadelphia.

The authors have presented zoologists and medical workers interested in the field of Parasitology with an indispensable aid in the classification of the Nematode parasites. Workers in this field have long been faced by the almost unsurmountable difficulty of a very widely scattered literature, often inaccessible and in difficult languages. In the case of isolated workers, especially if not expert in the field, this handicap has resulted in much duplication of work. The aid that such a key affords can hardly be overestimated and in itself alone fully justifies the authors for their painstaking and difficult task of compilation based on years of personal research.

The key itself does not attempt to place species but describes all the valid genera in terms of their type species and assigns them to natural groups even up to the class. It also furnishes an up to date list of all valid species appended to each genus, often with a list of suggested synonyms, and a bibliography of recognized species as well as keys for generic determinations. Aimless hunting through literature is practically eliminated and the isolated worker is guided in securing a short and very definite list of works from some library with the assurance of getting most if not all that he needs for his particular problem.

As in all unconsolidated fields, isolated efforts here have resulted in many individualistic attempts at a general classification that differ widely from each other. All of these have had their evident weaknesses and all their strong points. Yorke and Maplestone have had courage to place a new scheme in printed form. Regardless of the accuracy of their particular system, it at least serves as a focus and out of such a comparative study can and will develop a more unified system of classification such as has been developed for many of the other better known phyla.

Fragmentary studies have often led to false impressions as to the proper affinities of various forms and the field is too vast for any worker to compass properly even a majority of the known Nematode parasites. Yorke and Maplestone have made a very successful start on the problem in as far as they could make original studies or refer to well established researches of other modern workers and have corrected many outstanding weaknesses in the earlier and more fragmentary attempts at establishing the proper relationships between the various groups. Many of these groups had unfortunately been based upon types now lost to view and many of the so-called re-identifications of these "lost" forms have been inaccurate because of the indefiniteness of the original descriptions, such as, e. g., many of the species credited to Leidy. As a result any compilation must unavoidably contain many contradictory relationships. Future workers will find their task much simplified in correcting such mistakes by virtue of this book.

In establishing their system of classification the authors wisely abandoned the musculature as an important criterion, relying in most cases on other characters of more general access and constancy. The limitation of the term *bursa* to the caudal formation of the males of the super-family Strongyloidea is very well taken. In the Ancylostomidae, the sub-family Necatorinae (Lane, 1917) is given preference over the prior rights of Bunostominae (Looss, 1911) because of the doubtful status of the genus Bunostomum Railliet, 1902. It would seem wiser to retain the older name until the dispute is actually settled in favor of the most recent designation. A very useful suggestion is advanced regarding the tendency to make hair-splitting differences in bursal ray formulae the basis of generic separation. These differences are seldom of more than specific value, and even then may often times be questioned. As an example of this tendency the authors contrast the satisfactory and simple classification of the Trichoneminae, in which only two genera, Trichonema and Poteriostonum, depend on bursal formulae for differentiation, with the Trichostrongylidae in which not only several genera

but even groups of genera depend in the use of the key upon bursal characteristics for their recognition. Undue importance has perhaps been placed on the character of the bursa in recognizing the Pseudaliidae as a family separate from the Metastrongylidae, instead of as a sub-family, Pseudaliinae, in the latter family. Other characteristics do not support the necessity of such a step The wisdom of separating the Oxyurids from the Ascarids is also somewhat questionable in that the possession of a posterior esophageal bulb as distinct from a posterior bulb-like ventriculus, as in Dujardinia and Multicaecum (Ascaroidea) and the Kathlaniidae (Oxyuroidea), is difficult to establish. The authors recognize this difficulty but believe themselves justified in their step by other minor characteristics. The simpler, and, apparently at the present at least, the more accurate division would be based upon family differentiation within a super-family Ascaroidea. The handling of the groups within the Oxyuridae (as defined by the authors) is excellent with but the possible exception of Probstmayria and Oxysomatium. These two genera seem nearer the Kathlaniidae than they do the Oxyuridae on the ground of possessing esophageal characters very similar to those found in Falcustra, etc. The emphasis on the precloacal musculature in separating the Subuluridae from the Heterakidae and Kathlaniidae seems somewhat forced. The position of Cissophyllus as more closely related to the Kathlaniidae than to the Subuluridae is also open to considerable doubt. The recognition of the family Heterocheilidae as separate from the Ascaridae is possibly justified, but is as yet not entirely necessary unless extraordinary weight is placed on the presence of digestive diverticula. The reason for placing Ascaridia with the Ascaridae are likewise somewhat questionable in the light of present knowledge. The handling of the super-families Spiruroidea and Filaroidea, especially the latter, indicates much careful and painstaking research in a very obscure and difficult group and furnishes a thoroughly logical starting point for future investigations. The above suggestions and comments are not intended as destructive criticisms but have been offered with a desire to add to what has been started by the excellent and far-reaching efforts of these two English parasitologists.

The make-up of the book is pleasing, the illustrations, new, accurate and numerous, and the typographical errors are few and of very minor importance. One very unfortunate feature which may easily give rise to much confusion is the fact that the work was printed in Great Britain on December 12, 1925, but the American edition carries the date of 1926.

A Manual of the Parasitic Protozoa of Man. By Charles F. Craig, 569 pp., 95 figs. J. B. Lippincott Co., Philadelphia and London.

This imposing and attractive treatise on the human parasitic protozoa represents the results of many years of work on the part of one whose contributions to the subject entitle his findings to be viewed with great respect. A perusal of the pages demonstrates the justification of the hope that was held out by the name and reputation of the author. It covers the field more adequately from a medical standpoint than any other work available in English, and is sure to appeal strongly to workers in the field by virtue of its comprehensiveness and clarity.

After an introductory chapter on the structure classification, and biology of parasitic protozoa, four chapters are devoted to parasitic amoebae, six to parasitic flagellates, one to the coccidia, four to malaria organisms, and one each to sarcosporidia and ciliata. In addition the author gives a most valuable technical appendix and good indexes. Under each organism one finds information concerning the nomenclature and structure, as well as biological characteristics, methods for cultivating the type, geographical distribution, methods of transmission, infection of experimental animals, relations to disease, pathology, prophylaxis and diagnosis.

At the end of each chapter the author gives in brief form a list of the chief articles cited in that part of the text. These lists are admirably full and will be

of great assistance to those who desire to follow out the necessarily brief discussions of the text. The author's style is clear and attractive; his treatment of the work of others is worthy of commendation for its fairness. The illustrations while not numerous are in general good. It is, however, difficult to justify one as crude as Figure 65.

One admirable feature of the book is the introduction of closely related parasitic forms from lower animals and somewhat similar species from the coprozoic fauna, as well as the free living types which are often sources of confusion and even error in diagnosis. These features will appeal strongly to those teachers, practitioners, students, and health officials for whom the work is specifically written and are calculated to aid the worker in avoiding some of the numerous errors which have characterized work in this little known and difficult field. It is not too much to say that the care with which this work has been written will be an important factor in contributing to the advance in the handling of these organisms such as could not well be given by one of lesser training and experience.

The author comments on the fact that those organisms usually classed as Chlamydozoa have been intentionally omitted from consideration, as well as the Spirochaetes and Rickettsia, since he believes "there is a very considerable amount of evidence available that indicates all of these organisms are much more closely allied to the bacteria than to the protozoa." It is interesting to know that precisely contrary views have been expressed by other workers in this field in publications of very recent date and while it must be granted that the subject is still *sub judice,* yet on practical grounds, methods of culture and study, as well as habits and various biological details, naturally lead to their consideration by protozoologists and consequently indicate the advantage of their treatment in a work on parasitic protozoa.

Animal Parasites and Human Disease. By Asa C. Chandler. Third Edition. 573 pp., 254 figs. John Wiley & Sons, Inc., New York. Chapman & Hall, Ltd., London.

The new third edition of this well known work appears just four years after the publication of the second. It shows numerous minor changes and a few of a more extensive character. The latter concern the flukes, the hookworm, and some protozoan parasites. New views on the transmission of certain protozoal diseases and the role of the sand fly therein have also been introduced, but on the whole the work has not been greatly altered.

The changes have sometimes involved rather amusing consequences, such as the omission of figure 5 still referred to in the text. The reference to the parasite of scarlet fever (p. 191) is certainly out of date as are references to "the present war." It seems somewhat incongruous to find the text still uses clumsy antiquated fractions of an inch to denote microscopic measurements. The reviewer does not recall a similar usage in any modern work.

Insects and Disease of Man. By Carroll Fox. 349 pp., 92 figs. P. Blakiston's Son & Co., Philadelphia.

To the numerous works already existant on medical entomology the author has added one that possesses distinctiveness and merits commendation. The subject is vast and the literature most extensive. Dr. Cox has endeavored with fair success to bring within narrow compass the essentials for public health practice. The work is extremely condensed and meant to be studied rather than read. Part 1 covers in 21 brief chapters the systematic and morphological data on Insects and Arachnida, to which are added one chapter on rodents and one on technique. Part 2 treats of Diseases Among Human Beings Carried by Arthropods and is embraced under 14 chapters. It is remarkable that so much has been brought within a narrow compass without the omission of important topics. The book may be highly commended to students and field workers even though both groups will desire to supplement their studies by wider reading in individual cases.

Parasites of Swine. By Maurice C. Hall. The North American Veterinarian, Chicago, Illinois.

In a series of booklets under the title of Worm Parasites of Domesticated Animals, designed to furnish parasitologists and veterinarians with information in brief form on an evidently complex subject, Dr. M. C. Hall has written a treatise on the Parasites of Swine. It appears to be the first attempt to treat the parasites of this host in an independent work. The convenient form and limited compass of the book commend it to the man who desires a handy volume. To accomplish the result aimed at the author has omitted intentionally much of the data on anatomy and taxonomy ordinarily included in such works. Very valuable data are given on treatment, a field in which the author has made experiments and discoveries of outstanding importance.

The Anti-Malaria Commission of the Egyptian Government has published a most valuable and exhaustive study of the *Mosquitoes of Egypt* written by T. W. Kirkpatrick. It describes the structure of the egg, larval stages and adult, and discusses the systematic arrangement of all species found in Egypt. The ecological and biological data which follow are especially interesting and afford a firm basis for a thoroughly successful anti-malarial campaign. The author is convincing in his view that mosquitoes are not a necessary concomitant of agriculture as practiced in Egypt. The illustrations are abundant and good.

The section on Animal Parasitology from the 7th edition of Dr. E. R. Stitt's well-known textbook has been translated and published as the first Chinese edition by R. T. Shields. This work is certain to be of great value to Chinese medical students. Both the author and the translator are to be congratulated upon providing for its wider usefulness in this way.

Collected Addresses and Laboratory Studies. Compiled by R. T. Leiper. Volume I, 1924-5. London School of Hygiene and Tropical Medicine.

Under this title has been brought together a series of contributions that appeared originally in widely separated publications. The addresses concern education in public health and allied topics. The laboratory studies cover researches on various parasites from Protozoa to Arthropods, and on diseases caused by such organisms. Both human and veterinary medicine are involved, as well as comparative helminthology in the broadest sense. The variety no less than the amount of the work represented in the volume is striking evidence of the breadth and activity of the institution in which it was produced and reflects great credit on the director of the school, Dr. Andrew Balfour, and his staff.

NOTE

Following the tragic death of Dr. B. H. Ransom last September, his friends in the Helminthological Society of Washington formed a committee to consider a suitable memorial to him. Among other plans considered was the suggestion that one number of the Journal of Parasitology be devoted to a Ransom Memorial Number and that the papers for this number be contributed by Dr. Ransom's personal friends and associates. This suggestion was brought to the attention of the managing editor of the Journal, Dr. Henry B. Ward, and having been approved by him, it was arranged that the committee should take entire charge of the number in question. Under this arrangement the September number of the Journal will appear as the Ransom Memorial Number. This form of memorial is in addition to some memorial of a different sort now under consideration by the committee.

Maurice C. Hall, Chairman.
C. W. Stiles,
Eloise B. Cram, Secretary.
W. W. Cort,
H. J. Nichols.

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